JOURNAL OF FORENSIC SCIENCES

*J Forensic Sci,* January 2010, Vol. 55, No. 1
doi: 10.1111/j.1556-4029.2009.01233.x
Available online at: interscience.wiley.com

**TECHNICAL NOTE**

**CRIMINALISTICS**

*Rebecca Hoile,*[1,2] *B.Sc.; Connie Banos,*[3] *Assoc. Dip.; Michael Colella,*[3] *M.Sc.; Simon J. Walsh,*[4] *B.Sc. (Hons); and Claude Roux,*[2] *Ph.D.*

# Gamma Irradiation as a Biological Decontaminant and Its Effect on Common Fingermark Detection Techniques and DNA Profiling

**ABSTRACT:** The use of disease-causing organisms and their toxins against the civilian population has defined bioterrorism and opened forensic science up to the challenges of processing contaminated evidence. This study sought to determine the use of gamma irradiation as an effective biological decontaminant and its effect on the recovery of latent fingermarks from both porous and nonporous items. Test items were contaminated with viable spores marked with latent prints and then decontaminated using a cobalt 60 gamma irradiator. Fingermark detection was the focus with standard methods including 1,2-indanedione, ninhydrin, diazafluoren-9-one, and physical developer used during this study. DNA recovery using 20% Chelex extraction and quantitative real-time polymerase chain reaction was also explored. Gamma irradiation proved effective as a bacterial decontaminant with *D*-values ranging from 458 to 500 Gy for nonporous items and 797–808 Gy for porous ones. The results demonstrated the successful recovery of latent marks and DNA establishing gamma irradiation as a viable decontamination option.

**KEYWORDS:** forensic science, gamma irradiation, microbial forensics, latent fingermarks, DNA, bioterrorism

From the origins of the germ theory and the development of penicillin, scientists have spent centuries studying pathogenic microbes and their toxins to combat disease in humans, animals, and plants. The emergence of new biological species such as severe acute respiratory syndrome, and outbreaks of more traditional pathogens such as influenza are major milestones in our epidemiological history. Recently there has been an emergence of a new phenomenon, potentially more fearful and more challenging than these natural occurrences—bioterrorism.

The deliberate use, or threat to use, disease-causing organisms and their toxins against the civilian population, in furtherance of a political or social objective (1), has defined bioterrorism and opened forensic science up to the challenges of processing evidence contaminated with a biological weapon.

One of the greatest achievements in microbial science is the ability to identify down to the genomic sub-types, the identity of the target organism. While identification of the bioweapon is essential, processing of traditional forensic exhibits is paramount to gather intelligence and evidence that ultimately enable the reconstruction of the event under investigation and assist the identification of the perpetrator.

Typically a major crime scene can contain a number of critical pieces of evidence including (but not limited to): latent fingermarks, DNA, documents, hairs and fibers, and electronic evidence. The analysis of fingermarks from major crime scenes and disaster victim identification incidents still remains one of the most frequent and critical pieces of evidence. Fingermark detection and analysis has undergone tremendous advances since its introduction to crime scene investigation in 1892 (2). From simple powders (e.g., aluminum powder) to chemical (e.g., amino acid reagents) and physicochemical (e.g., cyanoacrylate [CA] fuming and vacuum metal deposition) treatments and enhancements of latent fingermarks from porous and nonporous surfaces are well established and reliable. Yet do the decontamination steps that are necessary to render the scene and exhibits safe to process destroy or diminish the crucial forensic evidence available?

History indicates that the use of biological pathogens as a weapon is not a new phenomenon, with examples of terrorist attacks from the Aum Shinrikyo, a religious cult based in Japan responsible for the sarin gas attack and attempts to release anthrax (3). Domestic groups and individuals such as the Bagwhan Rajneesh who used Salmonella to draw attention to their political objectives, the release of viable anthrax spores through the American mail by a rogue scientist and the continued threat from groups such as Al Qaeda demonstrates the past and future interest in biological weapons (4).

The release of biological threat agents such as *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Francisella tularensis* (tularemia), and *Variola Major* (small pox), and toxins such as *Clostridium botulinum* (botox), pose the greatest risk because of their

[1]Forensic Counter Terrorism and Disaster Victim Identification Unit, NSW Police Force, 1 Charles St Parramatta, New South Wales, Australia.
[2]University of Technology, Centre for Forensic Science, Sydney, New South Wales, Australia.
[3]Australian Nuclear Science & Technology Organisation, PMB 1, Menai, New South Wales, Australia.
[4]Forensic and Data Centres, Australian Federal Police, GPO Box 401, Canberra ACT, Australia.

Received 28 Aug. 2008; and in revised form 15 Dec. 2008; accepted 20 Dec. 2008.

ability to be effectively manufactured with limited treatment and to cause high mortality and great economic loss. While each biological agent poses different threats based on their unique characteristics and effects, *Bacillus anthracis* the causative agent of the disease anthrax, remains one of the biological agents of choice for use as a weapon. This is due in part to its ability to produce tiny endospores (DNA encapsulated by a thick protective polysaccharide) enabling it to remain viable for many years in the environment, resisting desiccation, UV light, and many disinfectants (5).

Gamma irradiation is widely recognized as an effective biocidal. It has long been used in the food industry to sterilize or reduce the microbial load of certain foodstuffs and is widely used in the sterilization of medical and pharmaceutical devices. The recommended dose for this application is between 25 and 40 kGy (6). Gamma irradiation can achieve cell inactivation via ionizing radiation generated from a cobalt 60 source. Gamma photons can penetrate substances and damage or inactivate biological material by direct damage to the cells' genetic material or indirect intracellular damage through the buildup of external radicals causing intracellular damage (7).

The *Bacillus* group is a large genus comprising 70 species with *B. cereus* group, *B. anthracis*, *B. cereus*, and *B. thuringiensis* identified as pathovars of a single species, with the primary differences between *B. cereus* and *B. anthracis* the presence of pXO1 and pXO2 plasmids (8). It is for this reason that many research projects including this one focus on nonpathogenic relative species such as *B. thuringiensis.*

The research described herein was conducted to determine the effectiveness of gamma radiation as an evidence decontamination option and the effects of this method on the detection of latent fingermarks and DNA from evidence contaminated with viable *Bacillus* spores. The consequence of successful decontamination prior to forensic analysis will be discussed based on the outcomes of these findings.

## Materials and Methods

The project was divided into three phases. Phase 1 set out to determine the effective dose required for successful destruction of viable spores from porous and nonporous items. These substrates included paper (A4 photocopy paper), cardboard (standard A4 artists cardboard), borosilicate glass (Livingstone™ microscope slides), and hard plastic (polycarbonate strips). Test items were contaminated with a known quantity of viable spores and subjected to gamma irradiation at set doses. Phase 2 set out to determine the effects of gamma irradiation, as a decontaminant, on a number of commonly utilized latent fingermark detection techniques and Phase 3 looked at the effects of gamma irradiation on the recovery of human DNA from porous items. A detailed description of the procedures for each phase of experimentation is provided below.

### *Phase 1*

*Effects of Gamma Irradiation on Spore Survival*—Sample Preparation: a powdered preparation of *Bacillus thuringiensis* var. *kurstaki* (BT) spores was prepared to the value of $6 \times 10^7$ spores/g. A 10-fold dilution series was prepared to determine approximate spore counts. A 10 μL aliquot of the stock solution was inoculated onto each test sample and allowed to air-dry. Test samples of A4 copy paper and artists cardboard were cut into small rectangles divided into two sections, glass microscope slides, small plastic strips were marked in two using a china graph pen, and each test sample was labeled with the corresponding irradiation dose. 10 μL of the stock spore solution was inoculated onto each test sample and allowed to air-dry. Test samples were prepared in triplicate, where possible, for each radiation dose to be tested. All test samples were placed into small zip lock bags labeled with the designated radiation dose. Control samples for each dose were inoculated with spores and used to determine the approximate recovery rate of viable spores prior to irradiation. Swabs were then collected following gamma irradiation from the test samples area of inoculation using moistened swabs plated onto blood agar (BA) plates. Quality control plates were also incubated to demonstrate media sterility. Colony counts for each test matrix exposed to gamma irradiation were transformed to logarithms ($\log_{10}$ values), and a plot of dose versus average surviving population was constructed. To determine the radiation dose required to inactivate spores by 90% of the population an average $D_{10}$-value was calculated as the value of the slope of the regression, in Grays (Gy).

*Cobalt 60 Gamma Irradiator*—Processing of samples was conducted at the Australian Nuclear Science and Technology Organisation. This facility is equipped with the Gamma Technology Research Irradiator (GATRI). The GATRI is a large walk-in irradiator with source strength of 1.29 kGy/h and source area of 960W × 600H.

All test samples were placed into various size zip lock bags labeled with the designated radiation dose and placed into the GATRI for exposure to the cobalt 60 source. Irradiations were carried out at ambient temperature (*c.* 19.5°C) at various dose rates; (a) 0.375, (b) 0.438, and (c) 1.432 kGy/hr (varying dose rates determined by the distance and setup of items from the source) to achieve the target doses of 200, 500, 1000, 1500, 2000, 2500, 3000, and 5000 Gy (refer Table 1). Previous experiments up to 40 kGy indicated no growth of bacterial spores (R. Hoile, unpublished observation). Fricke (ferrous ammonium sulfate) and ceric-cerous (ceric sulfate/cerous sulfate) dosimeters were used in the predetermined minimum and maximum dose zones. Dosimeters monitored each of the irradiations.

### *Phase 2*

*Effects of Gamma Irradiation on Latent Fingermark Recovery*—Sample Preparation and Fingermark Deposition: test samples were prepared in triplicate for each method of print recovery and for each gamma dose tested. Sheets of A4 photocopy paper (test 1 porous) and standard artists cardboard (test 2 porous) were prepared with a dilution series of latent fingermarks using three donors chosen at random observed as having good fingermark deposits, and a control set for each. Each test sample was labeled with the fingermark recovery technique that being either ninhydrin, 1,8-diazafluoren-9-one (DFO), 1,2-indanedione or physical developer.

Glass microscope slides (test 1 nonporous) and plastic polycarbonate strips (test 2 nonporous) were labeled with the gamma dose and the technique of development (CA or powder). A dilution

TABLE 1—*Irradiation dose rates based on dosimeter measurements for test samples.*

| Packet Target Dose (Gy) | Average Dose (Gy) | Exposure Time (min) | Dose Rate |
|---|---|---|---|
| 200 | 232 | 32.03 | a |
| 500 | 523 | 69.04 | a and b |
| 1000 | 1042 | 41.97 | c |
| 1500 | 1565 | 111.01 | *Accumulative dose, dose rates a and c |
| 2000 | 2100 | 83.79 | c |
| 2500 | 2623 | 152.83 | *Accumulative dose, dose rates a and c |
| 3000 | 3600 | 125.76 | *Accumulative dose, dose rate c |
| 5000 | 5600 | 209.5 | *Accumulative dose, dose rate c |

series of latent fingermarks were placed onto test samples by three donors and repeated in triplicate.

Control samples were prepared for both the nonporous and porous substrates for each of the development techniques. The control sets included samples which were not contaminated with spores and did not undergo decontamination with gamma irradiation. The presence of bacterial spores alone did not interact with fingermark recovery methods (results not shown). All test samples were subjected to doses in the range of 200–5000 Gy with 500 Gy increments (refer Table 1) and once at 40,000 Gy using both the GATRI cobalt 60 facility and smaller Gammacell depending on the dose required. Latent fingermark detection techniques were then applied to both test and control samples using the range of techniques described in Table 2.

*Fingermark Development Techniques*—Fingermark development techniques were undertaken at the NSW Police Force Forensic Services Group Fingerprint laboratory following standard operating procedures. The methods are outlined below and a summary provided in Table 2.

*Gray and Magna Black Powder*—Nonmagnetic black powder (Lightning Powder Company, Monash, ACT, Australia) was applied to glass and plastic items using a squirrel hair brush (Lightning Powder Company). The developed fingermarks were highlighted with the Polilight (model PL500; Rofin Australia, Dingley, Victoria, Australia) set to white light mode.

*Cyanoacrylate Fuming*—Cyanoacrylate fuming was applied to glass and plastic test samples. Fifteen drops of superglue (Loctite 406 Instant Adhesive) was added to a tray placed on the heating mat at the rear of the fuming cabinet (Foster and Freeman MVC5000). Water was placed into a tray at the base of the cabinet. The cabinet was set for a relative humidity of 80% for a time of 30 min. Test samples were removed and prints photographed.

*Ninhydrin*—A working solution of ninhydrin (final concentrations: 3% ninhydrin stock, 5% v/v isopropyl alcohol in 92% v/v 1-methoxyperflurobutane [HFE 7100]) was prepared by dissolving ninhydrin stock solution in klenasol/HFE 7100 (nonpolar solvent carrier). Test and control samples of paper were immersed in the working solution, allowed to air-dry, and subjected to indirect heat via an iron press for 10 sec. Fingermarks were left to develop overnight at room temperature and illuminated using the Polilight (model PL500; Rofin Australia) set to white light mode.

*1,8-Diazafluoren-9-One*—The paper test and control samples were immersed in a working solution of DFO (final concentrations; 0.035% 1,8-diazafluorenone, 2.5% dichloromethane, 6% methanol, 0.5% glacial acetic acid, and 90% HFE 7100), air-dried, and immersed again. Once dry they were exposed to indirect heat from an iron (Singer™; Elna Steam Press, Sydney, NSW, Australia) for 10 sec. Marks were observed with the Polilight lamp (530 nm) and photographed using an orange 590 nm barrier filter on a digital Nikon F3 camera (Lidcombe, NSW, Australia).

TABLE 2—*Fingermark recovery methods selected for use on test samples.*

| Reagent/Technique | Glass | Plastic | Paper | Cardboard |
|---|---|---|---|---|
| Cyanoacrylate ester | X | X | – | – |
| Black powder (nonmagnetic) | X | X | – | – |
| Ninhydrin | – | – | X | X |
| Diazafluoren-9-one | – | – | X | X |
| 1,2-Indanedione | – | – | X | X |
| Physical developer | – | – | X | X |

*1,2-Indanedione*—A working solution of indanedione was prepared by mixing 2.5 g 1,2-indanedione with 25 mL of acetic acid, 225 mL of ethyl acetate, and 2250 mL of HFE 7100. The test and control paper samples were immersed in the working solution, allowed to air-dry, and then immersed again. Once dry the samples were then subjected to indirect heat via the iron press (Singer™ Elna Heat Press) set at 165°C. Fingermarks were observed under the Polilight lamp (530 nm) and photographed using a 590 nm barrier filter.

*Physical Developer*—The physical developer solution was prepared as follows: (a) 100 mL Redox solution A (3% w/v ferric nitrate, 8% w/v ferrous ammonium sulfate, 2% w/v citric acid, 4% w/v detergent-surfactant stock solution in water) and (b) 5 mL of silver nitrate Solution B (20% w/v silver nitrate in water). After Maleic acid prewash and rinsing in water, the test and control samples for this technique were individually immersed in the physical developer solution until a silver-gray contrast was visible. Samples were then washed in water and allowed to air-dry. Developed marks were photographed with a digital Nikon F3 camera.

*Phase 3*

*Effects of Gamma Irradiation on DNA Profiling*—DNA Sample Preparation and Extraction: to test the potential impact of decontamination on DNA recovery from porous substrates, simulated case work samples were prepared using standard A4 copy paper. A series of dilutions were prepared from the control stock of human blood and 10 μL of the undiluted and diluted serum (neat, 1:2, 1:4, 1:8, 1:16, 1:32) was inoculated onto the test samples and allowed to air-dry. Test sheets were prepared for two methods of DNA extraction namely tape lifts via adhesive tape and extraction via physical emulsion of paper. A test sheet containing the dilutions of the DNA for both methods was prepared for each irradiation dose tested (500, 1000, 2000, 3000, 5000, and 10,000 Gy). A control sheet was also prepared which would not undergo gamma irradiation. Test samples were prepared in triplicate and exposed to a cobalt-60 gamma source for the nominated doses. Samples were kept at −20°C prior to extraction. All test DNA samples were extracted using the 20% Chelex® (Bio-Rad Laboratory, Regents Park, NSW, Australia) with Microcon® (Qiagen, Doncaster, Victoria, Australia) in accordance with the NSW Police Force standard operating procedures and manufacturers' instructions.

To maximize recovery of DNA from paper, tape lift samples were immersed in 690 μL of prepared extraction mix in a ratio of 10 mL extraction buffer : 500 μL Proteinase K, incubated for 1 h at 56°C, vortexed, then centrifuged for 30 sec. Paper tape lift fragments were placed into spin baskets and centrifuged at 5000 × *g* for 3 min and baskets were discarded. The 20% Chelex solution was added to the digested supernatant (ratio 150:690 μL) and tubes were incubated for 30 min at 56°C followed by 100°C for 10 min. Resulting liquid was spun at 5000 × *g* for 3 min and the supernatant was transferred to Microcon® tubes for final extraction of DNA using 100 μL of $T_{10}E_{0.1}$ buffer. The eluted liquid was stored at 4°C prior to DNA quantitation.

Paper cut-out samples containing whole blood were left to air-dry and sections of samples cut out and placed into 1.5 mL Eppendorf tubes. A total of 1 mL $H_2O$ was added to each tube and vortexed. Tubes were centrifuged briefly with most of the liquid discarded. A 450 μL aliquot of 5% Chelex was added to submerge each sample within the tube. Tubes were then incubated at 56°C dry incubation for 30 min.

The sample tubes were then incubated at 100°C dry incubation for 8 min. Tubes were vortexed and centrifuged at high speed for 3 min. The eluted liquid was stored at 4°C prior to DNA quantitation.

*Quantitative Real-time Polymerase Chain Reaction*—The amount of human genomic DNA was ascertained from each sample using Quantifiler® Human DNA Quantification kit, run on an ABI Prism™ 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Quantitation standards were prepared by serial dilution in $T_{10}E_{0.1}$ buffer (10 mM) using 200 ng/μL Human DNA Standard included in the kit. Standard curve dilutions were prepared ranging from 50 to 0.23 ng/μL in threefold increments. Quantifiler reactions were prepared using 10.5 μL of Primer Mix, 12.5 μL of polymerase chain reaction (PCR) Mix (Tris–HCl pH 8.0; 0.1 mM ethylenediaminetetraacetic acid; 20 μg/μL glycogen) and 2μL of either DNA extract or control. Samples were loaded into a Microamp® (Applied Biosystems) Optical 96-well reaction plate using optical adhesive covers.

Thermal amplification was conducted following manufacturer's instructions of 95°C for 10 min, 45 cycles of 95°C for 15 sec, and 60°C for 1 min. Each plate was set up with duplicate standards and duplicate test samples. Extraction and amplification negatives were also added to each experimental plate. Based on TaqMan™ (Applied Biosystems) probe technology the relative quantity of the target DNA was determined by analyzing the change in fluorescent signal produced during PCR amplification. The SDS software is used to obtain the fluorescence emission data to generate an algorithm. Results for DNA quantitation are reported in ng/μL when applied to the standard curve generated.

## Results and Discussion

### *Gamma Irradiation as a Biological Decontaminant*

The use of gamma irradiation to decontaminate a substrate refers to the removal of viable microbes or inactivation of the cells. This quality is deemed as the loss by the cells of their colony-forming ability when inoculated onto nutrient agar; cells that have lost their ability are said to be killed, inactivated, or rendered nonviable by the action of radiation (9).

Micro-organisms vary greatly in their radiosensitivities, with early research indicating bacterial spores are more radioresistant than vegetative cells, gram negative organisms are more sensitive than gram positives, and dried spore preparations more sensitive than hydrated spores (10). This is particularly relevant in the context of bioterrorism where preparation of powdered bacterial spore mix would more likely be used for the inhalation of an aerosolized product, yet once exposed to the environment the variable water content would depend on the partial pressure of the water vapor present in the atmosphere in which they are held. The use of powdered *B. thuringiensis* mix as a biological contaminant reflects both the genetic similarities and environmental conditions probable to the use of aerosolized *B. anthracis*.

The microbial load of the contaminated items tested was *c.* $6 \times 10^7$/g, with no growth recorded after 48 h on BA postexposure to gamma from 5 to 40 kGy (results not shown). A narrower range of 200 Gy to 5 kGy with 500 Gy increments was established to obtain a standard survival curve. Table 1 indicates the actual dose received by the test samples based on the source strength and distance of the samples from the source. A total of 216 microbial test samples were subjected to a range of doses calculated using both Ceric-Cerous and Fricke (ammonium sulfate) dosimeters with batch uncertainty calculated at ±3.5% and ±2.0%, respectively.

The effectiveness of gamma irradiation to reduce microbial load was calculated based on recovery of viable spores postexposure to nominated gamma doses. The test items were swabbed and BA plates inoculated, with colony counts conducted after 24 h incubation. Counts >300 CFU (colony-forming units) per plate were considered too numerous to count and serial dilutions were conducted at these lower doses to reach approximate bacterial counts for log graph calculations. Table 3 indicates a rapid reduction in viability on all four test matrixes with minimal growth occurring after exposure to 2500 Gy (2.5 kGy) and no growth recorded after exposure to 3000 Gy (3 kGy).

The dose survival data allow for the calculation of the decimal reduction dose or $D_{10}$ value, the radiation dose required to reduce the initial population by one log cycle or to 10% survival (or 90% killed) (11). The raw data values were converted into CFU/mL to achieve a logarithmic value, which was then plotted on a log graph used to calculate the *D*-values for each matrix.

The decimal reduction dose is calculated along the straightest section of the log curve using the *X*-axis values between two log points. Figures 1 and 2 were used to calculate the *D*-values of 808 and 797 Gy, respectively for paper and cardboard. It would be

TABLE 3—*Average colony-forming units (CFU) postdecontamination.*

| Approx dose (Gy) | Paper | Cardboard | Glass | Plastic |
|---|---|---|---|---|
| 200 | >300 | >300 | >300 | >300 |
| 500 | >300 | >300 | >300 | >300 |
| 1000 | >300 | >300 | >300 | 150 |
| 1500 | 85 | 65 | 180 | 20 |
| 2000 | 12 | 35 | 12 | 16 |
| 2500 | 2 | 5 | 1 | 2 |
| 3000 | 0 | 0 | 0 | 0 |
| 3500 | 0 | 0 | 0 | 0 |
| 5000 | 0 | 0 | 0 | 0 |

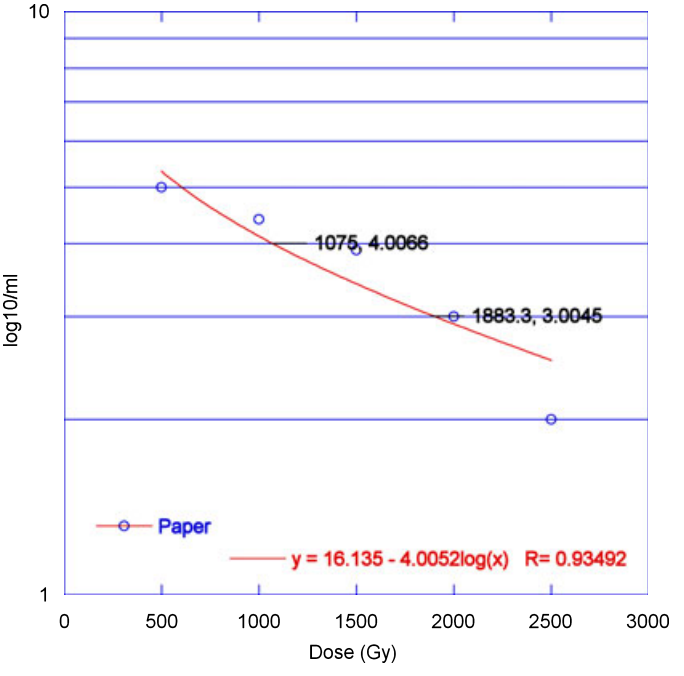


FIG. 1—*Log graph of spore survival on paper.*

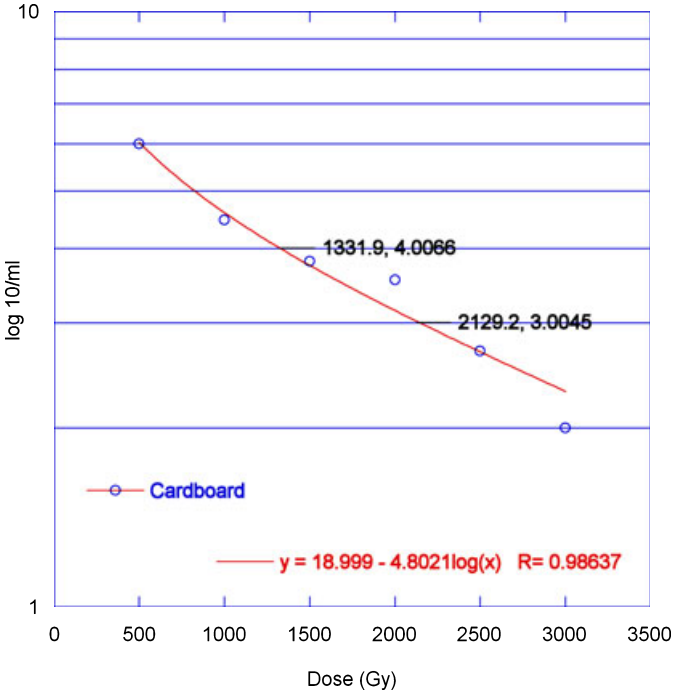


FIG. 2—*Log graph of spore survival on cardboard.*

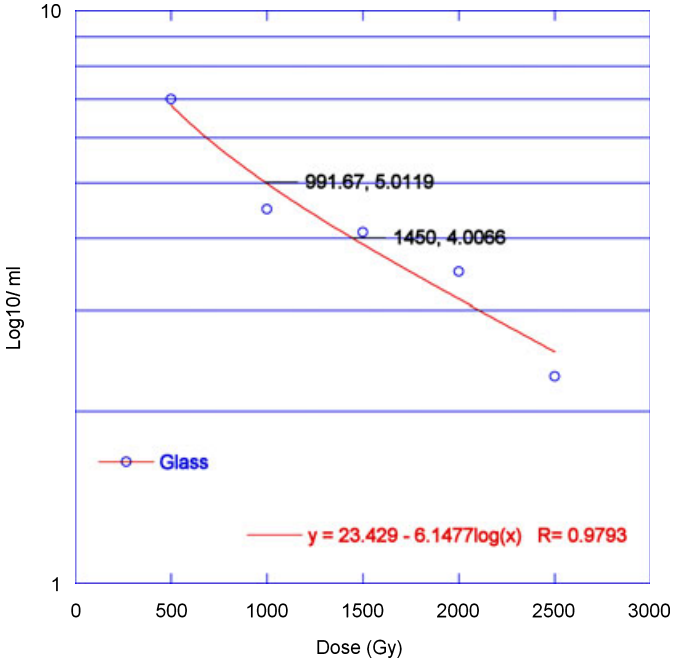


FIG. 4—*Log graph of spore survival on glass.*

expected that these two matrixes would have similar *D*-values because of their similar texture; interestingly the *D*-values for both glass (458 Gy) and plastic (500 Gy) are both similar and slightly lower indicating possible bacterial protection from the porosity of the paper and cardboard (refer Figs. 3 and 4). In contrast, the smooth and hard nature of the nonporous items provides less protection as bacterial spores remain on the surface.

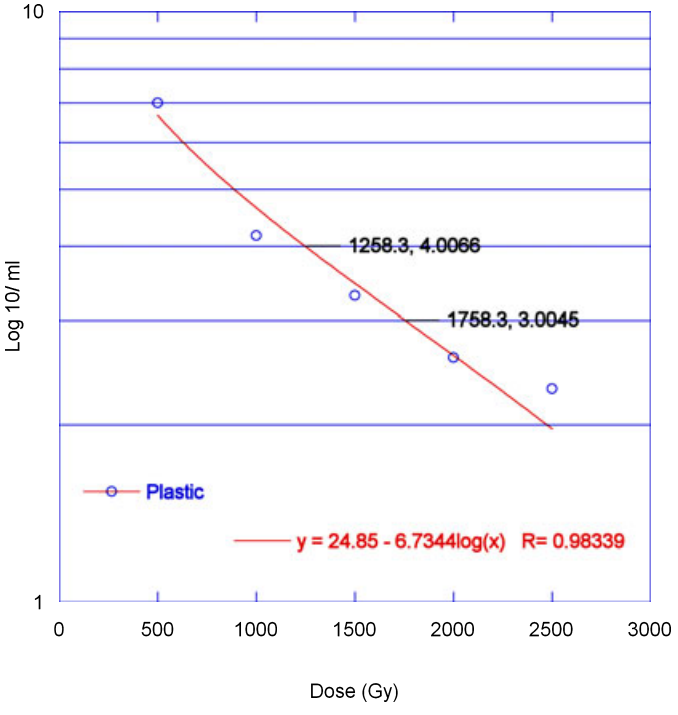


FIG. 3—*Log graph of spore survival on plastic.*

The $D_{10}$ value allows for an estimate of the number of powers of 10 that a population of the test species will be reduced by following exposure to the processing dose. This is made by dividing the value of the $D_{10}$ value into the dose used. For example a piece of evidence thought to contain *c.* $10^6$ spores/g would undergo a six log reduction after exposure to 3 kGy having a *D*-value of 0.5 kGy.

While the actual infectious dose for inhalational anthrax is estimated at being between 8000 and 10,000 spores, recent calculations based on the infections during the American release indicate this value to be much lower, due in part to our aging and immunocompromised communities (D. Beecher, personal communication). This means that a very small amount of anthrax spores could result in infection and consequently evidence contaminated with spores would need to be safely and effectively decontaminated prior to testing. Thus, this method could have been applied to the threatening letters used during the American anthrax attack which saw over 640 bags of mail examined with the most highly contaminated piece containing $8 \times 10^6$ CFU/g (12).

### *Effects of Gamma Irradiation on Recovery of Latent Fingermarks*

Fingermark development at a crime scene is not always possible because of the constraints of personal protective wear and equipment used in a contaminated environment. Laboratory-based detection techniques are also favored because of their increased sensitivity and effectiveness in a controlled environment. Therefore, it is necessary to have a means of decontaminating items removed from the scene prior to fingermark development to protect other forensic personnel and reduce the risk of further bacterial contamination. The range of fingermark techniques utilized in this research have been carried out as they would be within a laboratory subsequent to the hazardous material being present representing the standard practices for latent mark recovery postdecontamination and therefore no longer posing a risk to the operator.

*Nonporous*

Test samples of the nonporous items, glass (test 1) and plastic (test 2), were subjected to gamma irradiation with no detrimental effects observed for the plastic test samples. The glass test samples did however undergo increasing discoloration (clear to deep brown) which was visible from 1 kGy upwards, although the clarity was unchanged and did not affect recovery of latent fingermarks (refer Fig. 5). This colorimetric change is based on the properties of the glass interacting with the gamma creating visible absorption peaks (13). Both nonporous matrixes were subjected to fingermark recovery techniques postgamma including CA fuming and amido black powder. The recovery of prints from the test items were compared with the controls with no adverse effects to ridge detail and subsequent print quality.

FIG. 5—*Comparison of test and control mark on glass.*

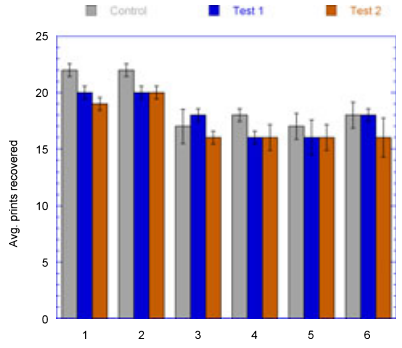


FIG. 6—*Effects of gamma irradiation on latent fingermarks.*

*Porous*

Porous items, paper (test 1) and cardboard (test 2), were subjected to gamma irradiation with no visible effect such as discoloration, friability, or ink damage within any of the doses tested.

Porous substrates were subjected to a range of latent print recovery methods for both amino acid and fatty-based prints including ninhydrin, 1,2-indanedione, DFO, and physical developer. Test samples were conducted in triplicate with the average recovery of quality prints indicated in Fig. 6. The quality of the prints was determined by the NSW Police Fingerprint Unit and only those that would enable identification were considered acceptable. The recovery of quality prints after exposure to 40 kGy indicates no detrimental effect to the constituents of latent prints enabling the recovery of vital evidence postdecontamination.

*Effects of Gamma Irradiation on Recovery of DNA Profiles*

Human DNA evidence has traditionally been recovered from blood, semen, and saliva and can also include trace of DNA from paper and even latent fingermarks (14). The collection of biological evidence is achieved at the crime scene with samples being sent to the laboratory for DNA extraction and analysis. Should the crime scene be contaminated with a biological agent it would not be possible to send these samples for DNA analysis and therefore the aim of this project was to establish a new system for the triage and treatment of DNA samples. In doing so, it also compared two commonly used extraction methods, namely direct emulsion and tape lifting. A small square of paper was inoculated with template DNA (blood) and exposed to a range of gamma irradiation doses. The DNA was extracted from the paper samples via both direct (removal via cutting of small inoculated square and extraction using Chelex® extraction) and indirect methods (tape lifting via adhesive tape, followed by Chelex® extraction). The direct method while more destructive to the paper, consistently recovered larger amounts of DNA from the porous test samples, as indicated in Table 4. Amplification of the extracted DNA from the neat sample generated an average value of 0.0364 ng/μL when adjusted to the standard curve, and was higher when compared with the indirect method providing 0.0258 ng/μL. This result is an indication of the complexities and randomness of the Chelex® extraction process and the percentage loss of DNA substrates during the lifting process. These factors must be taken into account when considering the plausibility of obtaining DNA from paper.

The results in Table 4 reflect the recovery of DNA from blood spots on paper exposed to 5000 Gy. A threat letter would be processed for DNA using either of the extraction techniques described with DNA recovered from the range of gamma doses tested, 500, 1500, 2500, 5000, and 10,000 Gy (results not shown). This

TABLE 4—*Average DNA quantitation values for blood on paper exposed to 5000 Gy.*

| Dilution Series | Direct Extracted DNA | ng/μL | Amt. Potentially Available in PCR* (ng) | Indirect Extracted DNA | ng/μL | Amt. Potentially Available in PCR* (ng) |
|---|---|---|---|---|---|---|
| Neat | 3.64e-002 | 0.0364 | 0.73 | 2.58e-002 | 0.0258 | 0.5 |
| 1:2 | 2.58e-002 | 0.0258 | 0.52 | 2.01e-002 | 0.0201 | 0.4 |
| 1:4 | 3.55e-003 | 0.00355 | 0.07 | 3.70e-003 | 0.0037 | 0.07 |
| 1:8 | 3.18e-003 | 0.00318 | 0.06 | 2.71e-003 | 0.00271 | 0.05 |
| 1:16 | 2.46e-003 | 0.00246 | 0.04 | 1.18e-003 | 0.00118 | 0.02 |
| 1:32 | 2.04e-003 | 0.00204 | 0.04 | 2.62e-003 | 0.00262 | 0.05 |
| NDC | NEG | 0 | | NDC | 0 | |

*Applying a maximum of 20 μL of template DNA per polymerase chain reaction (PCR; 50 μL total reaction volume).

indicates that the gamma dose required to reduce the viability of bacterial spores such as *B. anthracis* may affect the quantity of DNA recovered but does not exclude possible recovery of human DNA (blood) from paper. The low recovery levels may also be explained based on the selection of the extraction method. Research by Sewell et al. (15) has recently demonstrated that the use of different extraction kits can influence the recovery rates of DNA from paper.

This ability to recover DNA profiles postgamma irradiation correlates with work conducted at the Australian Nuclear Technology and Science Organisation, on the effects of gamma irradiation on the recovery of DNA profiles directly from blood (S. Abbodante, personal communication). This work illustrates that human STR profiling was not detrimentally affected when exposed to up to 10 kGy of gamma irradiation, using Applied Biosystems Amp*Fl* STR Profiler Plus system. In this study full profiles obtained from blood-stained items were still obtainable up to 50 kGy dose although degradation in the profile peak heights was observed between 10 and 50 kGy, with more severe degradation occurring after exposure to >50 kGy. Therefore, while further research is needed in this area, these combined experiments demonstrate successful bacterial decontamination is achievable at gamma levels (5–10 kGy) enabling the forensic investigator to process DNA samples postdecontamination.

The potential decontamination methods employed should be based on both the substrate and the type of evidence required, with no one decontamination solution able to meet all the needs of each forensic discipline. Therefore, other decontamination methods need to be explored. For example, work conducted by the Royal Canadian Mounted Police (16) focuses on the effects of decontamination in relation to chemical warfare agents on DNA. This work concludes that the decontamination process has a marked effect on DNA yet this does not compare, as the decontamination method is different as is the contaminant. Further work has been conducted on the effects of a biological decontaminant CASCAD® but again it is not comparable with this research.

Experimental work conducted prior to gamma irradiation explored the use of formaldehyde gas as an effective biological decontaminant with limited effects on latent fingermarks from nonporous material (17). Used in conjunction with gamma irradiation, an effective triage system for contaminated evidence can be established and the recovery of traditional forensic evidence achievable. Ultimately, forensic evidence and forensic procedures must be holistically assessed within the context of the case under investigation and appropriate analysis conducted based on prioritization.

## Conclusion

The advent of a bioterrorism event brings with it new challenges for the forensic disciplines including the collection, triaging, and analysis of contaminated evidence. This study has demonstrated how a gamma dose of >3 kGy can be used as a decontaminant for bacterial spores on paper, cardboard, glass, and plastic with no adverse effects on the recovery and analysis of latent fingermarks. This project also demonstrated an application to the recovery of human DNA from contaminated evidence such as paper, with the use of gamma irradiation having limited adverse effect on the recovery of human DNA and subsequent profile analysis. This experiment sought to establish decontamination methods for items contaminated with bacterial spores as a result of a biological release and not the sterilization of gross powders and liquids. The ability to decontaminate evidence such as a threat letter contaminated with anthrax spores at such a low gamma irradiation dose is vital for the forensic community, enabling these items to enter the evidence recovery cycle in an effective and efficient timeframe.

Future research will determine the effectiveness of gamma irradiation as a biological decontaminant on other forms of evidence such as electronic equipment and discuss the development of a contaminated evidence triage system.

## References

1. Budowle B, Schutzer S, Einstein A, Kelly LC, Walsh A, Smith J, et al. Building microbial forensics as a response to bioterrorism. Science 2003;301:1852–3.
2. Champod C, Lennard C, Margot P, Stoilovic M. Fingerprints and other ridge skin impressions. Boca Raton, FL: CRC Press, 2004.
3. Okumura T, Hisaoka T, Yamada A, Naito T, Isonuma H, Okumura S, et al. The Tokyo subway sarin attack—lessons learned. Toxicol Appl Pharmacol 2005;207:471–6.
4. Guillemin J. Biological weapons; from the invention of state sponsored programs to contemporary bioterrorism. New York, NY: Columbia University Press, 2005.
5. Anderson B, Friedman H, Bendinelli M. Microorganisms and bioterrorism. New York: Springer Science+Business Media, Inc., 2006;64–88.
6. Anthony P. Effects of gamma irradiation on Bacillus pumilus E601, a biological indicator for irradiation sterilization. Ann Arbor (MI): University Microfilms International, 1974.
7. Grieb T, Forng R, Brown R, Owolabi T, Maddox E, McBain A, et al. Effective use of gamma irradiation for pathogen inactivation of monoclonal antibody preparations. Biologicals 2002;30:207–16.
8. Blatchley ER III, Meeusen A, Aronson AI, Brewster L. Inactivation of *Bacillus* spores by ultraviolet of gamma radiation. J Environ Eng 2005;131(9):1245–52.
9. Ley FJ, Winsley B, Harboard P, Keall A, Summers T. Radiation sterilization: microbiological findings from subprocess dose treatment of disposable plastic syringes. J Appl Microbiol 1971;35:1.
10. Kumta US, Lewis NF. Sterilization by ionizing radiation. In: Proceedings of an International Atomic Energy Agency Conference, 1974 April 1–4; Vienna. Montreal, Canada: Multiscience Pty Ltd, 1975;37–60.
11. Mazzola PG, Penna TCV, Martins A. Determination of decimal reduction time (D-value) of chemical agents in hospitals for disinfection purposes. BMC Infect Dis 2003;3:24.
12. Beecher DJ. Forensic application of microbiological culture analysis to identify mail intentionally contaminated with *Bacillus anthracis* spores. Appl Environ Microbiol 2006;8:5304–10.
13. Lee HC, Gaensslen RE. Advances in fingerprint technology, 2nd edn. Boca Raton, FL: CRC Press, 2001.
14. Shalhoub R, Quinones I, Ames C, Multaney B, Curtis S, Seeboruth H, et al. The recovery of latent fingermarks and DNA using a silicone-based casting material. Forensic Sci Int 2008;178:199–203.
15. Sewell J, Quinones I, Ames C, Multaney B, Curtis S, Seeboruth H, et al. Recovery of DNA and fingerprints from touched documents. Forensic Sci Int Genet 2008;2(4):281–5.
16. Wilkinson DA, Sweet D, Fairley D. Recovery of DNA from exhibits contaminated with chemical warfare agents: preliminary study of the effect of decontamination agents and chemical warfare agents on DNA. Can Soc Forensic Sci J 2007;40:15–22.
17. Hoile R, Walsh SJ, Roux C. Bioterrorism: processing contaminated evidence, the effects of formaldehyde gas on the recovery of latent fingermarks. J Forensic Sci 2007;52:1097–102.

Additional information and reprint requests:
Rebecca J. Hoile, B.Sc.
Forensic Counter Terrorism and Disaster Victim Identification Unit
NSW Police Force Headquarters
Level 5 Tower B, 1 Charles Street
Parramatta
New South Wales 2150
Australia
E-mail: hoil1reb@police.nsw.gov.au